Message

From: Nadine Kotlarz [nkotlar@ncsu.edu]
Sent: 4/26/2018 1:23:05 PM
To: McCord, James [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=McCord, James]
CC: Detlef R. U. Knappe [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=user17c3f77b]; Jane Hoppin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=userebcfc262]; Lindstrom, Andrew [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=04bf7cf26aa44ce29763fbc1c1b2338e-Lindstrom, Andrew]; Strynar, Mark [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5a9910d5b38e471497bd875fd329a20a-Strynar, Mark]
Subject: Re: Update on serum method

Thanks James.
We did not add PFO2HxA to the calibration curve.

On Thu, Apr 26, 2018 at 7:49 AM, McCord, James <mccord.james@epa.gov> wrote:

> Responding to a couple things here for space.
>
> Detlef – We are observing legacy PFAS compounds in our blank samples that I am fairly certain I have isolated to the LC system, separate from the samples themselves. We were able to run blank serum with no detections just a few weeks ago but have picked up contamination in the meantime. The peaks are several million counts, so it vastly outstrips the low levels we are measuring in intensity and kills the sensitivity.
>
> Nadine – Regarding PFMOAA we have a running background from unrelated noise that makes it difficult to see low levels. Further, the retention time is in the instrument dead volume, which means the response is extremely sensitive to matrix effects, but we are able to observe a peak at ~1.2 minutes (see attached). In the blood it is difficult to determine whether any peaks observed in that region are the result of a compound or of noise without MS/MS confirmation, however I did notice a few samples that had minor peaks in that region. Quantitation would be highly suspect without changes to the chromatography and/or an SIL internal standard.
>
> Regarding PFO2HxA, if we extract the masses we observe peaks in blood as well as in the standard calibration curve, but not in SRM1957 (see attached). The elution time is in that heavily noise influenced dead volume region, which strikes me as slightly too early for PFO2HxA and I am inclined to say that we do not observed this compound conclusively.
>
> --
>
> James McCord

From: Detlef Knappe [mailto:knappe@ncsu.edu]
Sent: Wednesday, April 25, 2018 10:56 PM
To: Nadine Kotlarz <nkotlar@ncsu.edu>
Cc: Jane Hoppin <jahoppin@ncsu.edu>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; McCord, James <mccord.james@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>
Subject: Re: Update on serum method

Thank you, Nadine! Exciting indeed. When you say:

We see significant background for legacy PFAS in the blanks,

do you mean a noisy background from the matrix that interferes with legacy PFAS or legacy PFAS peaks are detected in the blanks?

Best,

Detlef

On Wed, Apr 25, 2018 at 9:30 PM, Nadine Kotlarz <nkotlar@ncsu.edu> wrote:

> Hi everyone,
>
> James, Mark and I have been working on the serum method using EPA's Orbitrap.
>
> Our method is 50 uL serum with formic acid denaturation and acetonitrile protein crash. After centrifugation to separate proteins, we load 100 uL of the acetonitrile fraction into an LC vial for analysis. Injection volume is 25 uL.
>
> We prepared standards in calf serum for PFMOAA, GenX, Nafion byproduct 1, Nafion byproduct 2, and the legacy PFAS at concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 15, 20, 25 ng/mL for each compound. We ran 10 randomly selected serum samples from the GenX Exposure Study and we ran SRM1957 to see if our measurement of PFAS in this material is close to what others have reported. We did not do internal standard correction for this run but would include internal standards in the future.
>
> Results are summarized in the summary tab of the excel spreadsheet. Calibration curves (with some points omitted for the different compounds) are in the ppt.

Initial observations:

1. GenX standard curve looks okay down to 0.5 ng/mL.

2. Poor response for Nafion byproduct 1 at the lower end of the cal curve (< 10 ng/mL)

3. For GenX and Nafion byproduct 1, the serum sample response is not significantly different from the blanks (calf serum).

4. We do not see PFMOAA in the samples or the standards (James, can you please confirm that we do not see it even in the 25 ng/mL?)

5. For Nafion byproduct 2, the mean concentration across the 10 serum samples is 3.4 ng/mL, and sample response is significantly higher than the blanks. The concentrations in the serum samples ranged from approx. 1-7 ng/mL.

6. Based on area counts, mean response for PFO4DA across the samples is 2 orders of magnitude above the blanks. We did not calibrate for this compound but Mark did get the standard from Chemours so we could reprepare standards with it.

7. Based on area counts, mean response for PFO5DoDA across the 10 samples is one order of magnitude above the blanks. We do not have a standard for this compound.

8. We see significant background for legacy PFAS in the blanks. For now, we do not know how to get rid of it. This background flattens the standard curves for PFHxS and PFOS and makes it difficult to quantify the legacy compounds in the serum samples.

9. In an interlab study of SRM1957, the reference values were 5 +/0 0.40 ng/mL PFOA and 21.1 +/- 1.2 ng/mL PFOS. We measured 4.2 ng/mL and 23.7 ng/mL PFOA and PFOS, respectively.

Please let us know your thoughts. Thanks,

Nadine